Investigation Preliminary antimicrobial and anticancer properties: on Topic Rubia tinctorum plant by using Polydimethylsiloxane (CAR/PDMS)

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Abstract

In this study, the antioxidant content in Rubia tinctorum was investigated. The extraction of essences is sensitive to operational conditions. Therefore, the effect of different extraction techniques by using HS -SPME fiber assembly Carboxen/Polydimethylsiloxane (CAR/PDMS), on the quality of essence oil composition was inspected and the composition of the final product was recognized using gas chromatography and mass spectroscopy.Essential Rubia tinctorum is widely used in pharmaceutical, sanitary, cosmetic, agriculture and food industries for their bactericidal, virucidal, fungicidal, antiparasitical and insecticidal properties. Their anticancer activity is well documented. This review is focused on the activity of essential Rubia tinctorum and their components on various types of Blood cancer cells. The chemical composition of the essential Rubia tinctorum from was analyzed by GC-MS. The main constituents were. Ocimene , Sabinene hydrate acetate, Bornyl acetate, Thymol , Methyl isoeugenol ,isoelemicin, Asarone, Neophytadiene. The cytotoxic effect of essential and extracts Rubia tinctorum were analyzed, the results showed that the substance. Good resistance against the toxicity of cell lines is shown MOLT4.

Keywords: Antioxidants, Gas chromatography, Rubia tinctorum, Blood cancer cells

1 Introduction

Antioxidants are chemicals that interact with and neutralize free radicals, thus preventing them from causing damage. Antioxidants are also known as "free radical scavengers."The body makes some of the antioxidants it uses to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants. Fruits, vegetables, and grains are rich sources of dietary antioxidants. Some dietary antioxidants are also available as dietary supplements [1,2] Examples of dietary antioxidants include beta-carotene, lycopene, and vitamins A, C, and E (alpha-tocopherol). The mineral element selenium is often thought to be a dietary antioxidant, but the antioxidant effects of selenium are most likely due to the antioxidant activity of proteins that have this element as an essential component (i.e., selenium-containing proteins), and not to selenium itself [3,4].

Many observational studies, including case–control studies and cohort studies, have been conducted to investigate whether the use of dietary antioxidant supplements is associated with reduced risks of cancer in humans. Overall, these studies have yielded mixed results [5]. Furthermore, the formation of cancer cells in the human body can be directly induced by free radicals.

Natural anti-cancer drugs such as chemopreventive agents in the treat-ment of cancer have gained in popularity. Studies demonstrated that fucoidan extracted from brown seaweed is a potential ROS scavenger and an important free-radical scavenger which is also capable of preventing oxidative damage [6,7] , and is therefore an important effector in the prevention of cancer. Radical scavenging compounds such as fucoidan from seaweeds can thus be used indirectly to reduce cancer formation in the human body. Several studies have reported that fucoidan has antiproliferative activity in cancer cell lines in vitro, as well as inhibitory activity in mice with tumors [8,9]. The anticancer activity of This herb has been used as carminative, laxative, stomach tonic, neuralgia relief, anti-inflammatory, antivirus, anti-parasite, antifungal and antibacterial (10,11). Phyto-chemical investigations lead to separation of Coumarins, alkaloids, Flavonoids and Terpenoides from different species of prangos. Further researches on aerial essences and seeds of Prangos ferulacea identified 33 types of oils. In the oil produced from the fruit, 39 compositions were determined which were mainly consisted of alpha-pinene. Monoterpenes, sesquiterpene and Coumarins were the other important compositions of the root.

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Antioxidants are the compounds protecting and decelerating the oxidation of other molecules and reducing oxidation speed with inhibition of free radicals, peroxide decomposition and chelating up the metals (12, 13, 14).

2 Materials and Methods

2.1 Materials

- Usual laboratory instruments and Clevenger using British Pharmacy standard.

- Circulation bath for constant temperature and as a condenser; type 8mLw made by mLwUH.

- Chemical analysis materials from Merck and Fluka with desired anhydrite sodium sulfate with 97% sincerity and normal hexane with 95% sincerity and standard oleic acid.

2.2 Instruments

- Gas chromatography and mass spectroscopy (GC, GC-MS): GC model HP-6890 made by HEWLETT PACKARD (USA)

- Mass spectroscopy Model HP-5973 made by HEWLETT PACKARD USA

- Gas chromatography and simultaneous gas chromatography and mass spectroscopy device (GC-MS)

- SPME fiber assembly excluding solid phase and SPME fiber holder made by SUPELCO (USA).

- Electric mill for herbaceous parts grinding, if necessary; this mill is needed for SPME method and the model is Ikawerke M20 (Germany).

Table 1. Characterisation of the used fiber in the SPME method

Type of fiber: (carboxen / poly dimethyl siloxane , CAR / PDMS)
Adsorbent thickness: 75 micrometer
Type of adsorbent connections: strongly network
Color: Black

2.3 Experimental procedure

2.3.1 Chromatography tests:

For Rubia tinctorum plant essence oil using Extract HS-SPME for (essential oil) methods. Analysis of the essential oil was performed using a Hewlett Packard 6890 GC equipped with a HP-5MS capillary column (30 m×0.22mm i.d., 0.25 μm film thickness) and a mass spectrophotometer 5973 from the samecompany for GC/MS detection with an electron ionization system energy (10 eV)was used. Helium was the carrier gas, at a flow rate of 1 ml/min., injector and detector MS transfer line temperature were set at 250 and 290 °C, respectively. Column temperature was initially kept at 60 °C for 5 min., then gradually increased to 220 °C at the rate of 6 °C/min.

2.3.2 Hydraulic distillation (HD) for (Extract) kelatin plant

The essence oil was gathered using Clevenger instrument. 100 gram of dried herb was grinded and the essence was gathered in the instrument for 3.5 hour. The operation performed using anhydrite sodium sulfate. The produced oil was conserved in a dim and closed container till analyses (see fig1).
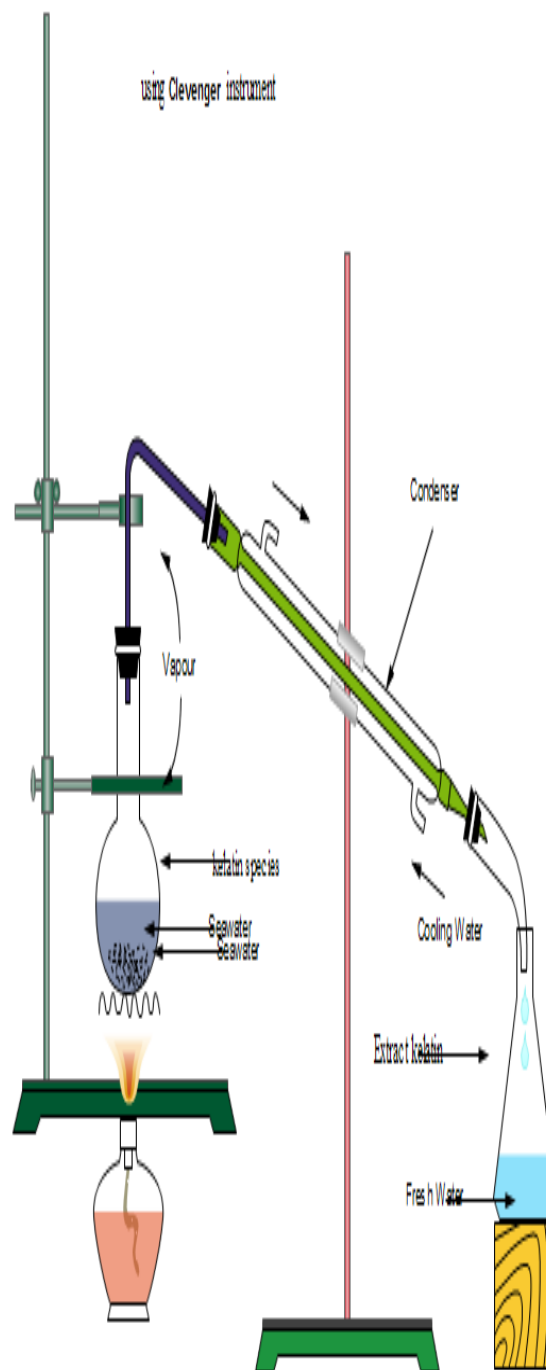

Figure 1. Schmatic of using HD method.

3 HS-SPME method

The method carried out using minimum amount of herb powder (1 g) without using any solvent. The circulating bath model mLw8 made by mLwHU was used in this experiment which had the ability of temperature control during extraction process. The fiber assembly was kept in a 10 ml glass container and the fiber holder attached to the container. The glass container was inside the circulating bath to reach the bath temperature. For heat desorption of pulled components on the fiber, the injection performed immediately into the GC-MS nstrument. in This study tests the suitability of the Carboxen–polydi- methylsiloxane (CAR–PDMS) fiber.The SPME device and CAR–PDMS (75mm) were used as fibers used in this study were purchased from internal standards (I.S.s). (results are shown as in table 1).The fibers ereMeSEt and Et S were supplied by Aldrich conditioned by inserting them into the GC system injector at 280 c for 30 min and they were immedi-were ately used to prevent contamination.Before the extraction with the fiber, the sample vials were equilibrated for 30 min at 25C. After- wards, the stainless steel needle in which the fiber is housed was pushed through the vial septum, allow- ing the fiber to be exposed to the headspace of the sample for 30 min. Then, the fiber was pulled into the needle sheath and the SPME device was removed from the vial and inserted into the injection port of the GC system for thermal desorption at 300 c for 1min.

4 Separation and identification of components in essence oils

As the components present in essence oils are known as volatile and semi-volatile oils, therefore GC-MS method was applied for separation and identification of the components. The result spectrums were compared with standard mass spectrum of Adams (Adams, R.P., 2004). In order to confirm the identified components of standard mass spectrum, Quatz deterrence index was applied. Firstly, the Alkanes of C8-C25 were injected into GC-MS and deterrence time for each Alkane was measured using KI=100n when 'n' is the number of carbons in related Alkane. Quatz deterrence index of essence oils were calculated using the following equation:

$$KI=100n + 100((t_x-t_n)/(t_{(n+1)}-t_n))   \quad (1)$$

After dewatering the produced oil, the oil was diluted using normal hexane (Merck) with the proportion of 1 to 10 and then injected into GC-MS.
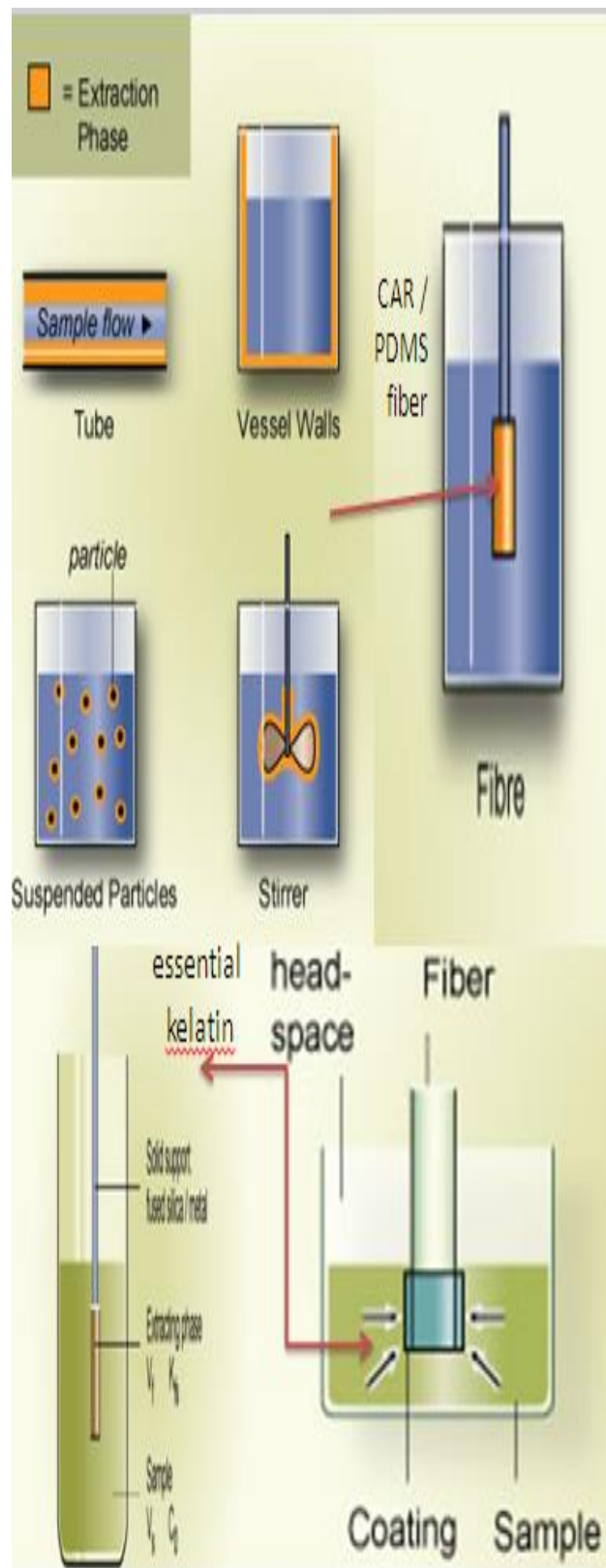

Figure 2. Fibre SPME modes: direct extraction.

The most widely used technique of sampling with solid phase microextraction onsists of exposing a small amount of extracting phase (coating) associated with a fibre to the sample, for a predetermined amount of time.

V_f = volume of fibre coating; K_{fs} = fibre/sample distribution coefficient; V_s = volume of sample; C_0 = initial concentration of analyte in the sample. Typically, the microextraction process is considered complete when the analyte concentration has reached distribution equilibrium between the sample matrix and the fibre coating. The equilibrium conditions can be described by equation (2), according to the law of mass conservation, if only two phases are considered (for example, the sample matrix and the fibre coating):

$$C_0 \cdot V_s = C_s^\infty \cdot V_s + C_f^\infty \cdot V_f \quad (2)$$

The distribution coefficient K_{fs} of the analyte between the fibre coating and sample matrix is defined as:

$$K_{fs} = \frac{C_f^\infty}{C_s^\infty} \quad (3)$$

Equations (2) and (3) can be combined and rearranged into:

$$C_f^\infty = C_0 \cdot \frac{K_{fs} \cdot V_s}{K_{fs} \cdot V_f + V_s} \quad (4)$$

Finally, the number of moles of analyte n extracted by the coating can be calculated from equation (5):

$$n = C_f^\infty \cdot V_f = C_0 \cdot \frac{K_{fs} \cdot V_s \cdot V_f}{K_{fs} \cdot V_f + V_s} \quad (5)$$

Equation (5) indicates that the amount of analyte extracted onto the coating (n) is linearly proportional to the analyte concentration in the sample (C0), which is the analytical basis for quantification using SPME.Equation (5), which assumes that the sample matrix can be represented as a single homogeneous phase and that no headspace is present in the system, can be modified to account for the existence of other compartments in the matrix, by considering the volumes of the individual phases and the appropriate distribution constants. In addition, when the sample volume is very large, i.e. $V_s \gg K_{fs} \cdot V_f$, equation (5) can be simplified to:

$$n = K_{fs} \cdot V_f \cdot C_0 \quad (6)$$

Which points to the usefulness of the technique when the volume of the sample is unknown. In practice, the fibre can be exposed directly to the flowing blood, ambient air, water, etc. The amount of extracted analyte will correspond directly to its concentration in the matrix, without depending on the sample volume. The amount of analyte extracted onto the fibre coating is at a maximum when the equilibrium is reached, thus achieving highest sensitivity. If sensitivity is not a major concern of analysis, shortening the extraction time is desirable. In addition, the equilibrium extraction approach is not practical for solid porous coatings, due to the displacement effect at high concentrations. For these circumstances, the extraction is stopped and the fibre is analyzed before the equilibrium is reached. The kinetics of absorption of analytes onto a liquid fibre coating can be described as

$$n = \left(1 - e^{-a \cdot t}\right) \cdot C_0 \cdot \frac{K_{fs} \cdot V_s \cdot V_f}{K_{fs} \cdot V_f + V_s} \quad (7)$$

Where t is the extraction time, and a is a time constant, representing how fast an equilibrium can be reached. When the extraction time is long, equation (7) becomes equation (5), characterizing equilibrium extraction. If the extraction equilibrium is not reached, equation (7) indicates that there is still a linear relationship between the amount (n) of analyte extracted onto the fibre and the analyte concentration (C0) in the sample matrix, provided that the agitation, the extraction time, and the extraction temperature remain constant. As equation (6) indicates, the extraction process is dependent on the distribution constant Kfs. This is a characteristic parameter that describes the properties of a coating and its selectivity toward the analyte versus other matrix components. Because of its solvent-free nature and the small size of the fibre coating, SPME can be interfaced conveniently to analytical instruments of various types. Only extracted analytes are introduced into the instrument, since the extracting phase is non-volatile and insoluble in most organic solvents. Thus, there is no need for complex injectors designed to deal with large amounts of solvents, and these components can be simplified for use with SPME. Depending on the method of subsequent analysis, the sensitivity of determinations using the SPME technique is very high, facilitating trace analysis. Although in most cases the analytes are only partly extracted from the sample, all extracted material is transferred to the analytical instrument, resulting in good performance. Carryover should be checked for each analyte and the desorption conditions should be chosen so that the analyte remaining on the fibre is less than 0.1% of the initial amount. The solvent free process results in narrow bands reaching the instrument, giving taller, narrower peaks and better quantification. (See fig 3)
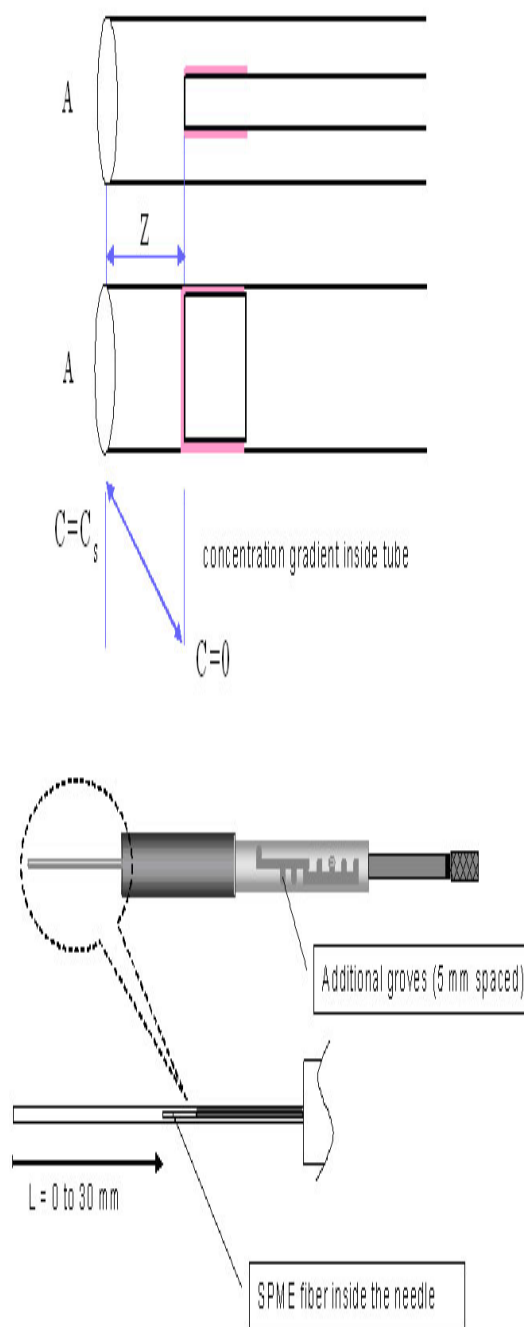
extracting solvents such as n-hexane (non-polar) and methanol were studied on the extraction of the essential oil from kelatin. Each time, the obtained essential oil was analyzed using Gas chromatography (GC). According to the obtained results of the previous studies and the beneficial effects of medicinal herbs, the aims of this study were extraction and investigation of the essential oil from Rubia tinctorum and evaluation of its effects to Inhibit blood cancer cells Growth. The results related to the essential oil are presented in the chromatograms. One of the active ingredients of essential oil is 2-methoxy-6-pentyl-1,4-dihydroxybenzene which is identified as having antimicrobial and anti-tumor properties.

6 The major components of the essential oil from Rubia tinctorum

The chemical composition of the essential oil from Rubia tinctorum was determined by gas chromatography spectrometer (GC). There were identified different compounds of the essential oil from this plant. The most important extracted compounds of the essential oil from chemical analysis by GC are shown in table 2 and the obtained Figure4. The maximum percentage of these compounds were related to bornyl acetate, Sabinene hydrate acetate, Ocimene, Thymol, Methyl eugenol, Methyl isoeugenol, Asarone, Neophytadiene, isoelemicin

Table 2. The most important constituents of the essential oil from Rubia tinctorum

Name	MF	FW	KI	Rt	%	%
Ocimene	$C_{10}H_{16}$	136	1050	5.4	9.64	4.165
Sabinene hydrate acetate<trans->	$C_{12}H_{20}O_2$	196	1256	12.38	10.01	4.234
Bornyl acetate	$C_{12}H_{20}O_2$	196	1288	13.37	8.77	3.789
Thymol	$C_{10}H_{14}O$	150	1290	13.79	8.02	3.463
Methyl eugenol	$C_{11}H_{14}O_2$	178	1403	18.22	10.77	4.646
Methyl isoeugenol	$C_{11}H_{14}O_2$	178	1492	21.79	8.09	3.494
isoelemicin<Z>, <E>	$C_{12}H_{16}O_3$	208	1570	24.74	8.25	3.564
Asarone<E>	$C_{12}H_{16}O_3$	208	1676	2893	100	43.182
Neophytadiene	$C_{20}H_{38}$	278	1841	34.08	13.58	5.866

Figure 3. Vf = volume of fibre coating; Kfs = fibre/sample distribution coefficient; Vs = volume of sample; C0 = initial concentration of analyte in the sample.

5 Data analysis

Study of the essential oil extraction by Clevenger method from Rubia tinctorum plantClevenger method was used for the isolation of essential oil as a traditional method of extracting essential oils from medicinal plants. The effects of different

Bornylacetate

isoelemicin<Z>, <E>

Methyl eugenol

Methyl isoeugenol

Neophtadiene

Ocimene
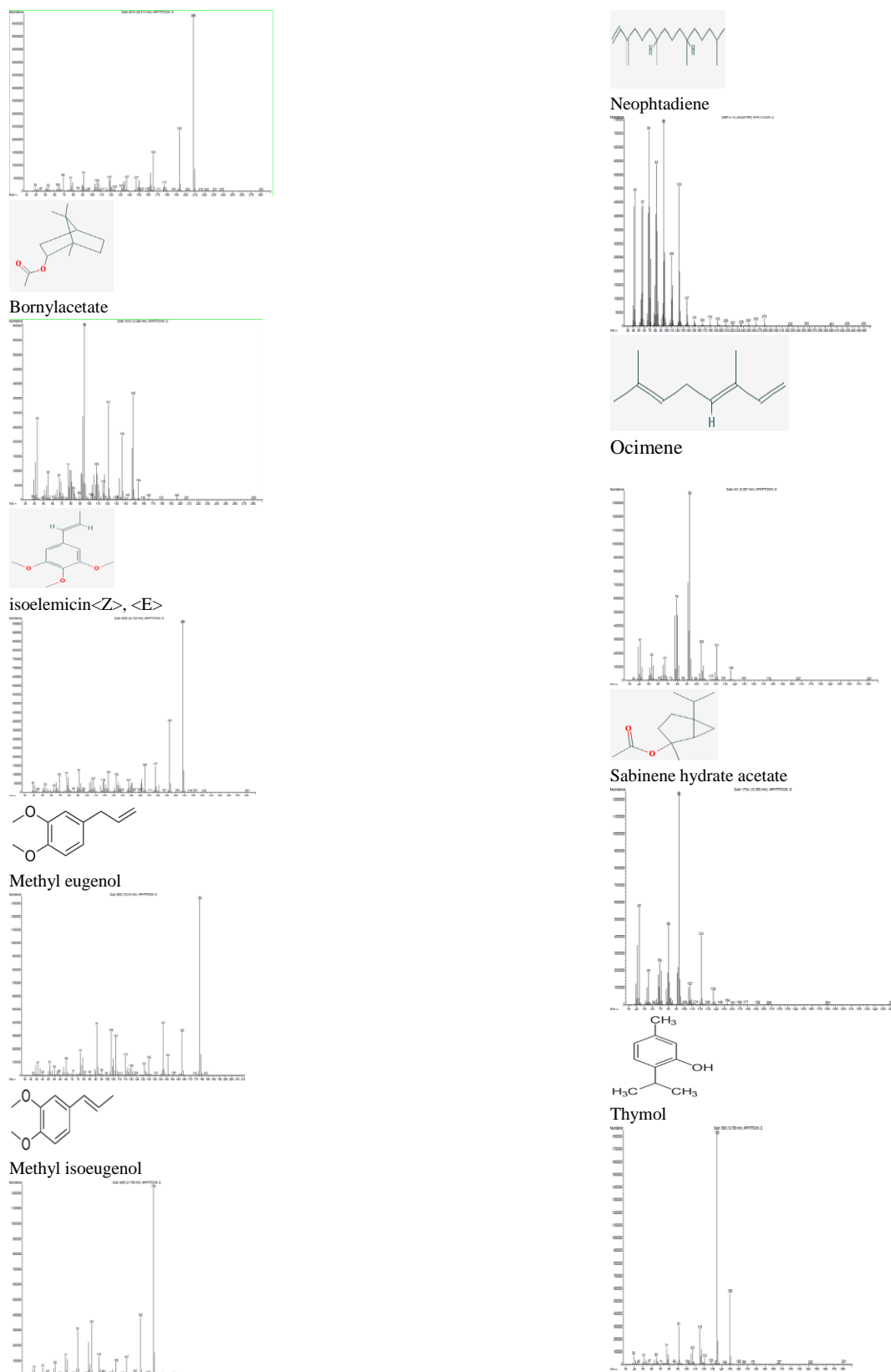

Sabinene hydrate acetate

Thymol

Figure 4. The mass spectra of compounds of the essential oil from Rubia tinctorum

7 Evaluation of the extracted essential oil from Rubia tinctorum

In order to measurement the percentage of the essential Oil components, hexan (non-polar solvent) and methanol a polar solvent was used for extraction of different compounds. The OBTAINED extracts were analyzed by liquid chromatography. According to the chromatograms, there are limited compounds in the extract. Figure 5 and 6 shows merely the obtained results and evaluation of these compounds was not provided due to lack the required standard.
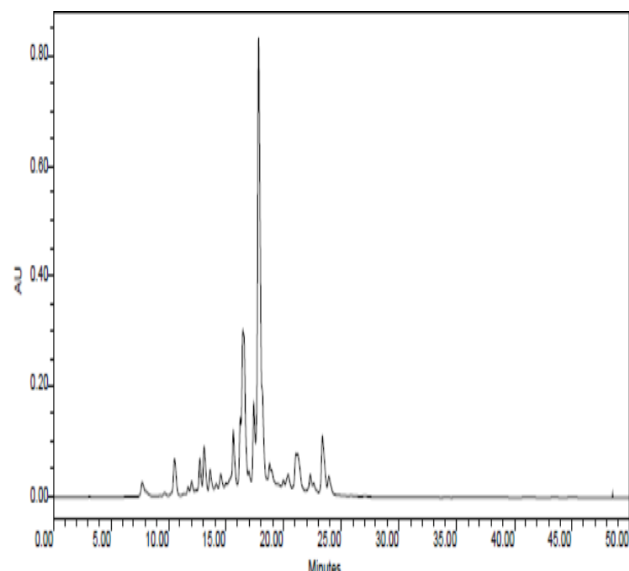

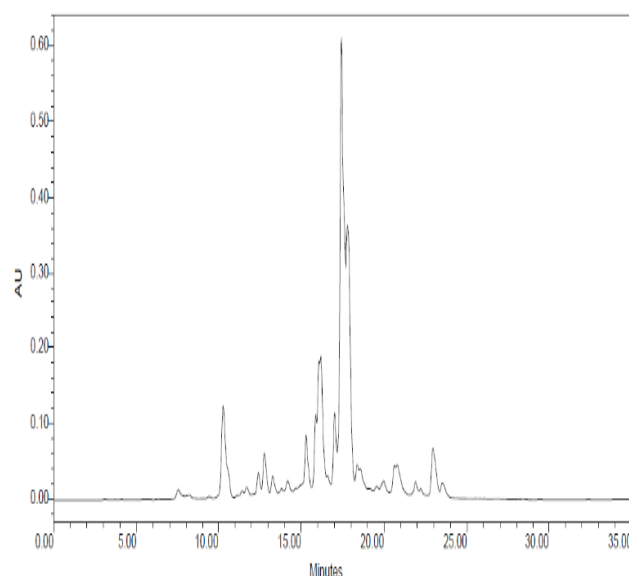**Figure 5.** The injected extracts of Rubia tinctorum

Figure 6. The injected essential oil from Rubia tinctorum

8 The results of cytotoxic effect investigation

Cytotoxic effects (LC50) of the essential oil and extract from Rubia tinctorum on the cell line (MOLT4v) have been shown the table 3 and 4 The results have been obtained by mean ± standard deviation .

Table 3. Cytotoxic effects of the essential oil from Rubia tinctorum

Essential	IC50μ)g/ml)
Mix	41 ± 0.0278
Cisplatin	5.1± 2.9057
Doxorubicin	17.7± 12.8819

Table 4. Cytotoxic effects of the extract from Rubia tinctorum

Extract	IC50μ)g/ml)
Aftimoon(hex+MeOH)	37.0±3.6
Mix (hex+MeOH)	25.48±3.3
Amfitoon (MeOH+H2O)	67.4±0.7
Cisplatin	7.0±2.4
Doxorubicin	5.4±0.3

The percentage of growth inhibition versus the concentration of plant extract and essential oil on the cell line (MOLT4v) have been demonstrated in the curves below.

9 Electrochemical study of antioxidant properties of essential oil from Rubia tinctorum

In this study, the electrochemical method was performed to evaluate the antioxidant properties of plant essential oil. Antioxidants act as reducing agents and oxidize on the surface of inert electrodes. Therefore, the relationship between the electrochemical behavior of molecules and electrochemical capacity is considered as the basis for the study. The antioxidant power will be increased with lower oxidation potential. The carbon electrode is commonly used as the working electrode to study the anti-oxidant behavior of medicinal essential oils. Cyclic voltammetry (cv) and differential pulse voltammetry (dpv) techniques were used for examining the electrochemical behavior of essential oil. In cyclic voltammetry, potential is applied within the specified limits the working electrode and the anode current resulting from the oxidation of chemical species is determined. Antioxidant properties of the samples were investigated by comparing with the properties of salicylic acid a standard reference. Other compounds were also used such as polyphenols chlorogenic acid, cordigol, cordigone, danthrone, 1,5-dihydroxy-3- methoxyxanthone, eriosematin, flemichin D, frutinone A, mangiferin, quercetin, 1,3,6,7- tetrahydroxyxanthone.The Oxidation potential of these compounds relative to the working electrodes is in the range from 0.4 V to 0.9 V. Considering that the redox potential of most compounds with antioxidant properties is in the potential range, kelatin essential oil can be introduced as a natural antioxidant. Voltammogram of the essential oil from kelatin in methanol has been shown in the figure below.The essential oil from kelatin has desirable antioxidant properties with regard to the obtained potential range, the

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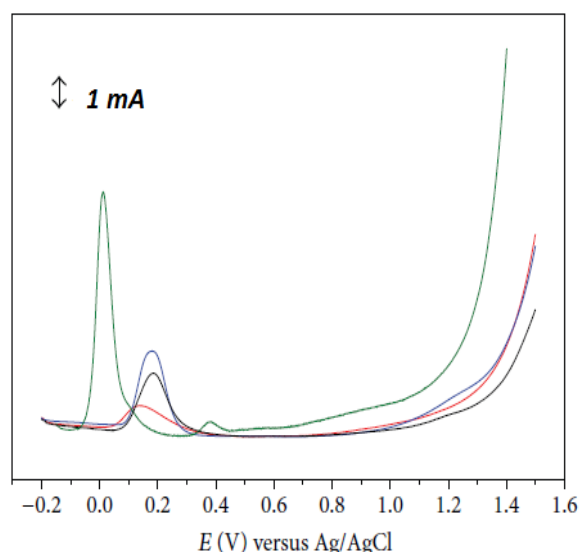intensity of the peaks and comparing it with common antioxidants (see fig 7).

Figure 7. Differential pulse voltammetry for 0.1 mM: black (Rubia tinctorum), Blue (benzoic acid), green (tri hydroxamic acids), red (hydrocaffeic acid)

Electrochemical methods have been often used for investigation of antioxidant activity of compounds, evaluation of antioxidant capacity and determination of electrochemical index. Various types of electrodes can be used for this assay purposes. Cyclic or differential pulse voltammetry are often used for these electrochemical measurements.The antioxidant capacity is one of the most important antioxidant parameters. The capacity is recognized as the ability of compound to prevent the oxidative degradation of other molecules. These methods are generally based on the direct reaction of study compounds with free radicals or on the reaction with transition metals. Spectrometric methods are often employed in the investigation of antioxidant properties. However, these methods are dependent on several parameters, such as temperature and time of the analysis.Electrochemical methods are rapid, simple and sensitive in the analysis of bioactive compounds and measurement of antioxidant capacity. Antioxidants can act directly as reduction agents and they tend to be quickly oxidized in aqueous solutions with inert electrodes. Therefore, the relationship between The electrochemical behavior of compounds with antioxidant activity and accordingly with their antioxidant capacity is very considered, because compounds with low oxidation potentials have higher antioxidant power. This fact is known that imbalance between The concentration of prooxidants and antioxidants can lead to oxidative stress and these changes are very effective in the pathophysiology of patients.

10 Antimicrobial and Anti-fungal effects of Rubia tinctorum plant

Containing graphene oxide has a different effect on the tested microorganisms. The results showed that this nanoparticle is effective on gram-positive and gram-negative bacteria and fungi, but depending on the type of microorganisms, its effectiveness varies. In Fig 8, the average diameter of the inhibition zones of Rubia tinctorum versus various microorganisms is expressed in millimeters. In this section according to the results of the disc diffusion method, fungi with an average diameter of the inhibition zones of the Cryptococcus neoformans are more sensitive to this nanoparticle compared to bacteria with an average diameter of the inhibition zones. Among the bacteria, Pseudomonas aeruginosa contains the largest average diameter of the inhibition zones (12.97 mm) and has a greater sensitivity to the nanoparticle than other microorganisms, while Salmonella arizona contains the smallest average diameter of the inhibition zones (6.41 mm). Among the fungi, Cryptococcus neoformans showed the largest average diameter of the inhibition zones (15. 22 mm).
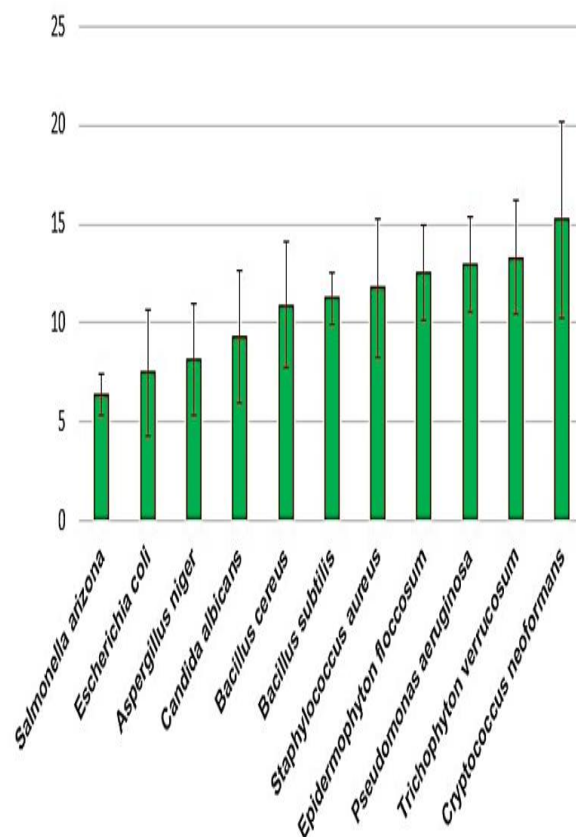

Figure 8. The average diameter of the inhibition zones of Rubia tinctorum

Considering the theoretical minimal bactericidal concentration, the lowest concentration of active ingredient, which 99% of bacteria has not grown in it, is considered as the effective concentration of active ingredient against

microorganisms. Since the size of the microbial inhibition zones does not precisely reflect minimum bactericidal concentration (MBC) and minimum inhibitory concentration (MIC), therefore, to determine the sensitivity of each microorganism to the antimicrobials, the determination of the diameter of the halo, MIC and MBC are required. In Table 5, the MBC and MIC of Rubia tinctorum containing graphene oxide, expressed in micro-liters, are plotted on 11 different tested microorganisms by the micro-broth dilution method. The results indicate that the Rubia tinctorum has a fatal effect in all concentrations on all tested microorganisms.see fig 6 and 9.
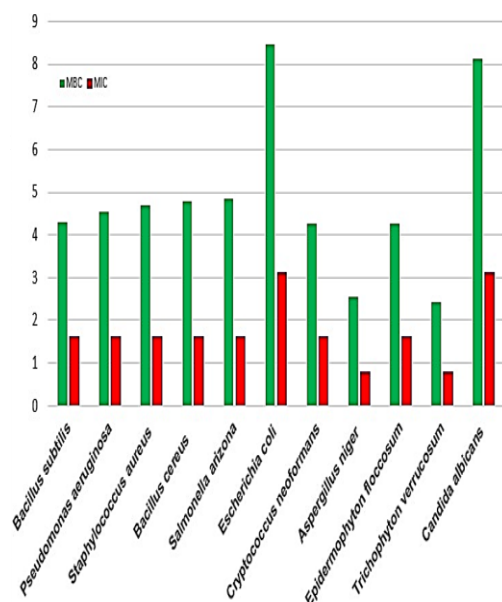

Figure 9. Minimum bactericidal concentration (MBC) and Minimum Inhibitory Concentration (MIC) of Rubia tinctorum.

According to the Figure (9), the microbes were classified into three groups of fungi, gram positive and gram negative bacteria, and MBC inhibitory growth at all levels of concentration were calculated in general. The results showed that E. coli MBC inhibitory growth was greater and there was a significant difference with other microorganisms. Also, based on this figure, gram positive bacteria (Staphylococcus aureus and Bacillus cereus) had the greater average diameter of the inhibition zone than gram negative bacteria and their differences were also meaningful.

11 Conclusion

Study the extraction compounds from native plants is very important due to the importance of medicinal plants in the last century and the attention of researchers to them as a safe natural resource. In Iran With various species of medicinal plants, an appropriate situation has been provided for these studies.

According to the obtained results from this study of Rubia tinctorum plant and the results of cytotoxicity tests, this plant has the ability and potential for medical or therapeutic uses especially for blood cancer cells. The results of analysis of the essential oil by gas chromatography and mass spectrometry showed presence of a variety of compounds in essential oils. It seems the extracted essential oils have a positive effects on cancer cells. Study the characteristics of each of these compounds could be a step towards developing the use of medicinal herbs. The extracted essential oils have a positive effect on cancer cells.

Ethical issue

Authors are aware of, and comply with, best practice in publication ethics specifically with regard to authorship (avoidance of guest authorship), dual submission, manipulation of figures, competing interests and compliance with policies on research ethics. Authors adhere to publication requirements that submitted work is original and has not been published elsewhere in any language.

Competing interests

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Authors' contribution

All authors of this study have a complete contribution for data collection, data analyses and manuscript writing.

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